Acute exercise increases the ventricular arrhythmia threshold via the intrinsic adenosine receptor system in conscious hypertensive rats

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Collins, Heidi L., and Stephen E. DiCarlo. Acute exercise increases the ventricular arrhythmia threshold via the intrinsic adenosine receptor system in conscious hypertensive rats. Am J Physiol Heart Circ Physiol 289: H1020–H1026, 2005. First published May 6, 2005; doi:10.1152/ajpheart.00156.2005.—Coronary artery occlusion—induced tachyarrhythmias that culminate in ventricular fibrillation are the leading cause of death in developed countries. The intrinsic adenosine receptor system protects the heart from an ischemic insult. Thus the increased functional demands made on the heart during exercise may produce protective adaptations mediated by endogenous adenosine. Therefore, we tested the hypothesis that a single bout of dynamic exercise increases the ventricular arrhythmia threshold (VAT) induced by coronary artery occlusion in conscious hypertensive rats via the intrinsic adenosine receptor system. To test this hypothesis, we recorded the VAT before and on an alternate day after a single bout of dynamic treadmill exercise (12 min/min, 10% grade for 40 min). A single bout of dynamic exercise significantly reduced postexercise arterial pressure (Δ–24 ± 4 mmHg) and increased VAT (Δ+1.95 ± 0.31 min). Adenosine receptor blockade with the nonspecific adenosine receptor antagonists theophylline or aminophylline (10 mg/kg) attenuated the cardioprotective effects of a single bout of dynamic exercise. Results suggest that strategies that increase myocardial ATP requirements leading to adenosine production provide protection against coronary artery occlusion.

Cardioprotective strategies

The cardiovascular response to dynamic, whole body exercise places profound demands on the heart. For example, cardiac output can increase four- to fivefold. The increase in cardiac output is primarily mediated by an increase in heart rate (HR) and to a lesser extent by an increase in stroke volume. The increase in stroke volume is primarily mediated by an increase in end-diastolic volume (Frank-Starling mechanism) and, in part, to a reduction in end-systolic volume (increased contractility mediated by adrenergic stimulation). These cardiovascular responses markedly increase myocardial oxygen consumption and accordingly, coronary blood flow can increase four- to fivefold (46). There is a tight relationship between myocardial oxygen consumption and coronary blood flow that suggests that products of metabolism mediate local control of coronary blood flow. Adenosine, a breakdown product of ATP, has been proposed as the major mediator of local coronary blood flow (3). Specifically, adenosine is the vasodilatory metabolite that links coronary blood flow to myocardial metabolism (3).

Adenosine also has an important role as an endogenous determinant of ischemic tolerance by modulating several components of cardiac function (6, 41). For example, adenosine has potent cardiac electrophysiological effects, including a negative chronotropic action on the sinus node and a predominant negative dromotropic action on the atrioventricular (AV) node (48). Adenosine therefore is used as an antiarrhythmic agent for the management of paroxysmal supraventricular tachycardia and other arrhythmias mediated by reentrant mechanisms involving the AV node (8–10, 32). Aminophylline, a nonspecific adenosine receptor antagonist, may have a clinical use in the treatment of ischemia-induced, adenosine-mediated brady-systole (26). Importantly, coronary artery occlusion-induced tachyarrhythmias that culminate in ventricular fibrillation are the leading cause of death in developed countries. Because increases in metabolism stimulate adenosine release and increase cardiac interstitial concentration of adenosine (11, 14, 27), an exercise-induced endogenous release of adenosine may provide protection from coronary artery occlusion-induced ventricular arrhythmias.

An understanding of how the heart responds to coronary occlusion and the role of exercise and the intrinsic adenosine receptor system in cardioprotection may provide strategies for developing novel therapeutic agents in the setting of an ischemic insult. Therefore, we tested the hypothesis that a single bout of dynamic exercise increases the ventricular arrhythmia threshold (VAT), induced by coronary artery occlusion, in conscious hypertensive rats via the intrinsic adenosine receptor system. Conscious, chronically instrumented rats were studied to negate the confounding effects of anesthetic agents and surgical trauma (22, 37). The clinical significance of coronary occlusion was hypothesized to document the effects of exercise and the intrinsic adenosine receptor system on cardioprotection.

METHODS

Design. This study was designed to test the hypothesis that a single bout of dynamic exercise increases the VAT induced by coronary artery occlusion in conscious hypertensive rats via the intrinsic adenosine receptor system. To test this hypothesis, the ECG, pulsatile arterial pressure (AP), and HR were recorded continuously during left main coronary artery occlusion under four experimental conditions. Specifically, the VAT was determined on a control day (No exercise, n = 7), after a single bout of dynamic exercise (Postexercise, n = 6), after a single bout of dynamic exercise with nonspecific adenosine receptor blockade (Postexercise Ad-X, n = 6), and after No exercise with nonspecific adenosine receptor blockade (No-exercise Ad-X, n = 5). The order of the experimental protocols was randomized, and each experimental protocol was separated by at least 1 wk.

Surgical procedures. All surgical and experimental procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee and conformed to the American Physiological Society’s “Guiding Principles in the Care and Use of Laboratory Animals” (4). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Animals.” All surgical procedures were performed by using aseptic surgical procedures. Rats were anesthetized with pentobarbital sodium (45 mg/kg ip) and supplemental doses (10–20 mg/kg ip) were administered if the rat regained the blink reflex or responded during the surgical procedures. The hearts were approached via a left thoracotomy through the fourth intercostal space. A coronary artery occluder, made from 5.0-gauge atraumatic prolene suture (8720H, Ethicon), which passed through a polyethylene-50 guide tubing (Clay Adams), was passed around the left main coronary artery 2 to 3 mm from the origin by inserting the needle into the left ventricular wall under the overhanging left atrial appendage and bringing it out high on the pulmonary conus (4). The guide tubing with the other end of the occluder was then exteriorized at the back of the neck. The tubing was filled with a mixture of Vasoline and mineral oil to prevent a pneumothorax and plugged with an obturator. At least 1 wk was allowed for recovery (25). During the recovery periods, the rats were handled, weighed daily, and acclimatized to the laboratory, treadmill, and the investigators. Subsequently, the animals were anesthetized as described above, and three insulated stainless steel ECG electrodes were sutured subcutaneously on the ventral side of the thorax. In addition, a telemetry device (Data Sciences International PhysioTel PA-C40) was implanted as previously described (4). The sensor of the telemetry device, located within the tip of the catheter, was inserted into the abdominal aorta for continuous nontethered recording of pulsatile arterial blood pressure via radiotelemetry. At least 1 wk was allowed for recovery (25). Again, during the recovery periods, the rats were handled, weighed daily, and acclimatized to the laboratory, treadmill, and the investigators.

Experimental procedures. Four experimental protocols (randomized and separated by at least 1 wk) were required to test the hypothesis that a single bout of dynamic exercise increases the VAT via the intrinsic adenosine system in conscious hypertensive rats: 1) No exercise, 2) Postexercise, 3) Postexercise Ad-X, and 4) No-exercise Ad-X. The order of the experimental trials was randomly selected. For the No-exercise trial, conscious unrestrained rats were studied in their home cages (~13,350 cm³). Rats were allowed to adapt to the laboratory environment for ~1 h to ensure stable hemodynamic conditions. Subsequently, the left main coronary artery was temporarily occluded by pulling up on the suture around the left main coronary artery (4). A rapid change in the ECG (ST segment elevation or depression), as well as a reduction in systolic AP (Fig. 1), documented coronary artery occlusion. The occlusion was maintained until the onset of ventricular tachycardia but no longer than 6.5 min to prevent myocardial damage (50). If the time to sustained ventricular tachycardia exceeded 6.5 min, the occlusion was stopped, and 6.5 min was used as the VAT. Normal sinus rhythm appeared on termination of the occlusion by gently compressing the thorax. In the rare event when the animal did not resume normal sinus rhythm, cardioversion was achieved (after the rat lost consciousness) with the use of one shock (10 J) of direct current. The VAT was defined as the time from

Fig. 1. Analog recording of arterial pressure (AP), ECG, and heart rate (HR; bpm, beats/min) immediately before occlusion of the left main coronary artery and at the onset of sustained ventricular tachycardia in an intact, conscious, hypertensive rat. Coronary artery occlusion (arrow) resulted in a rapid change in the ECG (ST segment elevation or depression) and decrease in AP. Occlusion was maintained until the onset of sustained ventricular tachycardia but no longer than 6.5 min to prevent myocardial damage. Sustained ventricular tachycardia was associated with rapid, wide QRS complexes and with decrease in AP.
coronary artery occlusion to sustained ventricular tachycardia resulting in a reduction in AP (Fig. 1).

For the Postexercise trial (at least 1 wk later), conscious unrestrained rats were again studied in their home cages. Rats were allowed to adapt to the laboratory environment for ~1 h to ensure stable hemodynamic conditions. Subsequently, the rats ran on a treadmill, without aversive stimuli, at 12 m/min, 10% grade for 40 min. By using this relatively low workload, without aversive stimuli, as well as providing training sessions, we feel that we are truly studying the response to exercise rather than a response to stress. Measurements of ECG, AP, and HR were recorded continuously during the single bout of dynamic exercise. After exercise, the rats were returned to their home cages. Twenty minutes after exercise, when postexercise hypotension and sympathoinhibition are evident (7, 24), the VAT, induced by coronary artery occlusion, was determined as described above.

The No-exercise and Postexercise trials were repeated (at least 1 wk later) after adenosine receptor blockade with the nonselective adenosine receptor antagonist theophylline or aminophylline (10 mg/kg). Aminophylline is a generically available, nonspecific adenosine receptor antagonist; however, its actions in vivo are consistent with a selective adenosine A1-receptor antagonist (47). Although specific adenosine receptor antagonists have recently been developed, they are not yet approved for clinical use. Therefore, to enhance the clinical significance of this study, aminophylline was used. The effective blocking dose of aminophylline was determined in five rats. Adenosine (25 μg/kg) produced depressor and bradycardic responses (Fig. 2, Δ−42 ± 6 mmHg and Δ−111 ± 15 beats/min). Aminophylline, 70 min after administration, reduced the responses to adenosine (Fig. 2, Δ+6 ± 1 mmHg and Δ−24 ± 9 beats/min).

Determination of ischemic zone. Three days after the conclusion of the studies, the rats were euthanized with an overdose of pentobarbital sodium. To determine the size of the ischemic risk area, the heart was excised with the occluder intact and perfused via the aorta with 30 ml of 0.9% saline to wash out the blood. Subsequently, the suture around the left main coronary artery was tied. Evans blue dye (300 μl, 1 mg/ml in 0.9% saline, Sigma Chemical) was perfused via the aorta causing the dye to infuse into the nonischemic area of the heart, leaving the ischemic regions unstained. The heart was trimmed leaving only the right and left ventricles, rinsed to remove the excess blue dye, and then weighed. The heart was trimmed again leaving only the ischemic region. The weight of the ischemic zone was expressed as the percentage of total heart weight (4, 5, 51).

Subsequently, to determine whether the occlusion produced a myocardial infarct, the heart was sliced transversally into ~1.0-mm sections and incubated in a 1% solution of 2,3,5-triphenyltetrazolium chloride (TCC, Sigma) at 37°C for 20 min. The heart sections were placed between two glass slides and immersed in 10% formalin overnight to enhance the contrast of the stain. TCC staining differentiates viable tissue by reacting with myocardial dehydrogenase enzymes to form a brick red stain. Necrotic tissue that has lost its dehydrogenase enzymes does not form a red stain and shows up as pale yellow (4). This stain has been shown to be a reliable indicator of myocardial infarction (15).

Fig. 2. Analog recording of the AP and HR responses to adenosine (25 μg/kg) before (A) and after (B) 70-min nonspecific adenosine receptor blockade with aminophylline (10 mg/kg). Adenosine produced depressor and bradycardic responses (Δ−42 ± 6 mmHg and Δ−111 ± 15 beats/min). Seventy minutes after administration, aminophylline reduced the responses to adenosine (Δ+6 ± 1 mmHg and Δ−24 ± 9 beats/min).
**RESULTS**

Figure 3 presents MAP (A) and HR (B) before (preexercise), during (exercise), and after a single bout of dynamic exercise (postexercise) during the Postexercise and Postexercise Ad-X trials. As expected, postexercise MAP was significantly lower than the preexercise value during the trials. There were no differences in MAP between trials. Thus adenosine receptor blockade did not alter the MAP response during or after exercise. Adenosine receptor blockade did not alter preexercise HR; however, HR was significantly higher during exercise and after exercise in the Postexercise Ad-X trial (Fig. 3B).

Figure 4 presents the VAT, induced by coronary artery occlusion, during the four experimental trials. In the No-exercise trial, the VAT induced by the occlusion of the left main coronary artery was 3.84 ± 0.23 min. Ad-X in the No-exercise condition (No-Exc Ad-X) did not alter the VAT (4.25 ± 0.27 min). Importantly, a single bout of dynamic exercise increased the VAT (Post-Exc, Postexercise; 5.71 ± 0.31 min). Nonselective Ad-X with aminophylline or theophylline (10 mg/kg) in the Postexercise trial (Post-Exc Ad-X) decreased the VAT to 4.37 ± 0.16 min. *P < 0.05, Post-Exc vs. No-Exc, No-Exc Ad-X, or Post-Exc Ad-X.

Table 1 presents MAP and HR immediately before the occlusion (preocclusion) and immediately before the onset of ventricular arrhythmia (prearrhythmia) across the experimental trials (Table 1).

### Table 1. MAP and HR immediately before occlusion of left main coronary artery and immediately before onset of ventricular arrhythmias under four experimental conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP</th>
<th>HR</th>
</tr>
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<tbody>
<tr>
<td>Preocclusion</td>
<td>163 ± 9*</td>
<td>142 ± 5</td>
</tr>
<tr>
<td>Prearrhythmia</td>
<td>151 ± 5*</td>
<td>122 ± 4</td>
</tr>
<tr>
<td>VAT</td>
<td>152 ± 6*</td>
<td>117 ± 4</td>
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</tbody>
</table>

*Values are means ± SE; MAP, mean arterial pressure; HR, heart rate; No-Exc, no exercise; Post-Exc, postexercise; No-Exc AD-X, no exercise following blockade of adenosine receptors; Post-Exc AD-X, postexercise following blockade of adenosine receptors. Preocclusion, before occlusion of left main coronary artery; prearrhythmia, before onset of ventricular arrhythmia. *P < 0.05, Preocclusion vs. Prearrhythmia; †P < 0.05, No-Exc vs. Post-Exc; ‡P < 0.05, No-Exc vs. Post-Exc AD-X.
interaction for MAP. These results indicate that MAP was significantly lower in the Postexercise trials. In contrast, MAP was not different between the No-exercise and Postexercise trials following blockade of adenosine receptors. In addition, preoclusion MAP, independent of treatment, was significantly higher compared with prearrhythmia MAP. The two-way ANOVA also revealed significant group and treatment effects without a significant group by treatment interaction for HR. These results indicate that preoclusion HR, independent of treatment, was significantly lower compared with prearrhythmia HR. Finally, the ischemic zone averaged 55 ± 4% of the total heart weight, and there were no signs of myocardial infarction.

**DISCUSSION**

In this study, a single bout of dynamic exercise resulted in a postexercise reduction in MAP. These results are consistent with several studies (7). Furthermore, the postexercise responses were associated with an attenuated hemodynamic response to coronary artery occlusion (Table 1) and increased VAT values (Fig. 4). Similarly, Babai et al. (1) documented that a single bout of treadmill exercise reduced the severity of life-threatening arrhythmias, induced by coronary artery occlusion in anesthetized normotensive dogs 24 h after the exercise bout. However, anesthetics have confounding influences on the cardiovascular system, including direct chronotropic effects, activation of the renin-angiotensin system, and/or activation of cardiovascular reflex phenomena (23, 28, 36). We studied conscious chronically instrumented rats to negate the confounding effects of anesthetic agents and surgical trauma (22, 37). Furthermore, we documented that the protective effects of exercise are manifest as early as 20 min after the exercise bout and occur in a model susceptible to life-threatening arrhythmias. Therefore, we have extended previous findings by documenting that a single bout of dynamic exercise increases the VAT in conscious hypertensive rats.

Nonselective adenosine receptor blockade with aminophylline prevented the postexercise cardioprotection (Fig. 4). Although the suggested pharmacological effects of aminophylline include the antagonism of adenosine receptors, the inhibition of phosphodiesterase, and the direct and indirect effects on intracellular calcium concentrations, adenosine receptor antagonism is believed to be the dominant mechanism of action of aminophylline when administered in therapeutic doses (47). In fact, aminophylline actions in vivo are consistent with adenosine A1-receptor antagonists (26, 47). The results from the current study are consistent with adenosine A1-receptor antagonists. Specifically, blocking A1 receptors would prevent adenosine-mediated, negative chronotropic action on the sinus node and negative dromotropic action on the AV node and would decrease the VAT, as observed in this study (Fig. 4). Coronary vascular adenosine A2A receptors may also be involved. Blockade of coronary artery A2A receptors would prevent potent coronary artery vasodilation (1, 9) and would decrease the VAT, as observed in this study. In contrast, blocking systemic vasodilator A2A receptors would prevent vasodilation and eliminate a reflex sympathoexcitation and thereby increase the VAT. Because aminophylline decreased the VAT, the results are consistent with the observation that aminophylline actions in vivo are mediated via local adenosine receptor antagonism. These results suggest that exercise-induced endogenous release of adenosine protects against coronary artery occlusion-induced tachyarrhythmias.

The story of adenosine in the cardiovascular system began in 1929 with Drury and Szent-Györgi’s discovery (12) that extracts from various tissues containing adenosine produced bradycardia, hypotension, and coronary vasodilation. During normoxia in the myocardium, adenosine is released in small amounts at a constant basal rate (44) and is present at low levels due to its rapid removal by three pathways. An imbalance between myocardial oxygen supply and demand results in the net breakdown of ATP, and adenosine release can increase up to 50-fold (31). Upon reaching the blood, adenosine is rapidly taken up in the red blood cells and is degraded. The half-life of adenosine in blood is very short (0.6–1.5 s) (29). Thus endogenous adenosine acts locally at the site where it is produced (44). These facts support a cardiac A1 receptor and coronary artery A2A receptor effect because endogenous cardiac adenosine would not be expected to mediate peripheral vasodilatation. In fact, a 5-min intravenous infusion of adenosine (140 μg·min⁻¹·kg⁻¹) did not alter blood pressure in healthy men (40).

The effects of adenosine are mediated by its interaction with specific G protein-coupled adenosine receptors. Adenosine activates acetylcholine-sensitive K⁺ current in the atrium, sinus, and AV nodes, resulting in the shortening of action potential duration, hyperpolarization, and the slowing of normal automaticity. Thus adenosine has negative chronotropic and dromotropic effects (2). Adenosine also inhibits the electrophysiological effects of increased intracellular cAMP, which occur with sympathetic stimulation. Because adenosine reduces calcium currents, it can thereby be antiarrhythmic by inhibiting delayed afterdepolarizations elicited by sympathetic stimulation. Adenosine also antagonizes the response to adrenergic stimulation (30, 44). For example, adenosine acts presynaptically to inhibit the release of norepinephrine (38). In 1985, Ely et al. (14) documented that adenosine exerted direct cardioprotective effects on the myocyte during ischemia and reperfusion. Finally, transgenic mice overexpressing adenosine A1 receptors have an increased resistance to ischemia (17). Thus adenosine has a profound cardioprotective effect during imbalance of myocardial oxygen supply and demand.

It is important to note that 50 million Americans are hypertensive or are taking antihypertensive medications (21). The morbidity and mortality associated with cardiovascular disease, including ventricular arrhythmias, increase exponentially with increasing levels of AP (18). In the United States alone, acute ventricular arrhythmias are associated with over 450,000 sudden deaths each year (19). Therapeutic strategies designed for primary prevention of ventricular arrhythmias are of limited or no success (20, 39). In fact, the Cardiac Arrhythmia Suppression Trial demonstrated an adverse outcome for many therapeutic strategies (13). Thus interventions, such as exercise, that increase the VAT have the potential to improve the lives of thousands of individuals.

**Limitations.** In this study, adenosine receptor blockade with the nonselective adenosine receptor antagonists theophylline or aminophylline attenuated the cardioprotective effects of a single bout of treadmill running. The use of a nonselective adenosine receptor antagonist should be considered because these xan-
thine derivatives are neither selective antagonists for adenosine nor selective for any of the four adenosine receptor subtypes (A1, A2A, A2B, A3). With regard to the receptor subtypes, A2B and A3 receptors are documented to be minimally affected by methylxanthines. Therefore, A1 and A2A receptors are likely the main targets for theophylline and aminophylline (16). Activation of A1 receptors mediates negative chronotropic effects (slowing of HR) and negative dromotropic effects (slowing and blocking AV nodal conduction) and inhibits norepinephrine release from cardiac sympathetic nerves. Inhibition of norepinephrine release is mediated through presynaptic adenosine A1 receptors (38). These effects of adenosine A1 receptor-mediated activation reduce myocardial oxygen consumption.

Activation of adenosine A2A receptors mediates potent coronary artery vasodilation (2, 33). Coronary artery vasodilation improves coronary blood flow and increases myocardial oxygen supply. Thus adenosine may be antiarrhythmic by reducing myocardial oxygen consumption (A1 receptor effect) and increasing myocardial oxygen supply (A2A receptor effect). Importantly, Stark et al. (45) documented a profound antiarrhythmic effect for adenosine that was blocked by theophylline. In addition, specific A1 receptor agonist 2-chloro-N6-cyclopentyladenosine produced comparable antiarrhythmic effects as those observed in the presence of adenosine alone. The authors concluded that the antiarrhythmic effects of adenosine are directly modulated by the A1 receptor (45). In contrast, Schreleck and Richardt (42) concluded that endogenous adenosine is antiarrhythmic during ischemia via the actions on A2A receptors. Thus the precise mechanism and receptor responsible for adenosine-induced cardioprotection are unknown. It is important to note that many receptor agonists and antagonists previously thought to be highly selective are in fact capable of interacting with multiple receptor subtypes at concentrations typically used. Furthermore, there are significant species differences in the pharmacology of adenosine receptors. These facts have added to the confusion regarding the precise receptor mediating the cardioprotective effects of adenosine. Future studies using more specific and selective A1 and A2A antagonists are needed to better define the role of adenosine receptors on exercise-induced increase in the VAT.

The limitation regarding the multiple actions of xanthine derivatives should also be considered. However, although there are several proposed mechanisms of xanthine-induced physiological and pharmacological effects, a large body of evidence suggests that adenosine receptor antagonism is the most important factor responsible for most pharmacological effects of methylxanthines in doses that are administered therapeutically.

Finally, there is mounting evidence that adenosine causes several types of ischemia-related bradysystoles (34). Importantly, it has been documented that aminophylline abolishes these bradysystoles that are refractory to atropine and epinephrine (26, 35, 49). Thus aminophylline appears to be a clinically relevant antagonist to block the cardiac effects of endogenous adenosine.

In summary, a single bout of dynamic exercise produced a postexercise reduction in AP and increased the VAT in hypertensive rats. The mechanisms responsible may involve, in part, the intrinsic adenosine receptor system.

GRANTS

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REFERENCES

ADENOSINE AND CARDIOPROTECTION


